**Transcriptomic Mapping of the 5-HT Receptor Landscape**

**Roberto De Filippo¹ and Dietmar Schmitz¹²³⁴⁵**

¹ Charité Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health; Neuroscience Research Center, 10117 Berlin, Germany.   
² German Center for Neurodegenerative Diseases (DZNE) Berlin, 10117 Berlin, Germany.   
³ Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität Berlin, and Berlin Institute of Health, Einstein Center for Neuroscience, 10117 Berlin, Germany.   
⁴ Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität Berlin, and Berlin Institute of Health, NeuroCure Cluster of Excellence, 10117 Berlin, Germany.   
⁵ Humboldt-Universität zu Berlin, Bernstein Center for Computational Neuroscience, Philippstr. 13, 10115 Berlin, Germany.

\* Correspondence to: roberto.de-filippo@charite.de

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# Abstract

Serotonin (5-HT) plays a pivotal role in regulating a wide range of brain functions, including mood, anxiety, sleep, and cognition. This study presents a comprehensive transcriptomic analysis of the 5-HT receptors (Htrs) transcription across approximately 4 million brain cells, utilizing single-cell RNA sequencing (scRNA-seq) data from the Allen Institute. We report on the differential expression patterns of all 14 known Htr subtypes, revealing a wide diversity in their prevalence and distribution throughout the brain. Notably, we found that 65.84% of cells transcribe RNA of at least one Htr subtype, with significant variability in prevalence across different depth of classification levels and distribution across brain regions. Htrs were found to colocalize in the vast majority of cases. The transcription patterns of Htrs can accurately inform a random forest classifier to identify specific classes and types of neurotransmitter-releasing cells with surprising success. Leveraging a a multiplexed error-robust fluorescence in situ hybridization (MERFISH) dataset provided by Harvard University we analysed the spatial distribution of each Htr confirming previous findings and uncovering novel patterns of possible expression at an unprecedented detailed level. Our findings underscore the complexity of the 5-HT system even at the single-cell dimension and provide new insights into the receptor-mediated mechanisms that underpin diverse neural functions and behaviors. To aid the exploration of the dataset at different level of granularity we provide a custom online visualizer.

# Introduction

# Results

**Htrs transcription overview**

We analysed the single-cell scRNA-seq dataset provided by the Allen Institute {Yao, 2023 #2828} focusing on the transcription of Htrs RNA across approximately 4 million brain cells. The scRNA-seq dataset comprehensively encompassed all known 14 Htr subtypes. 65.84% of cells transcribed RNA of at least one Htr. Prevalence of Htrs across the entire dataset was considerably different ranging from 0.09% of Htr3b to 34.26% of Htr1f (Figure 1a). RNA of 6 Htr was found in less than 2.5% of the cells (Htr1d, Htr2b, Htr3a, Htr3b, Htr5b, Htr6). On the other hand, RNA of Htr1f, Htr2a and Htr2c was present in at least 1 every 5 cells. Average amount of RNA transcription also varied across receptors (Supplementary Figure 1a). Interestingly, the variation in amount of RNA shared around half (r²=0.55) of the variability with the prevalence, i.e., genes found in more cells also tended to be transcribed more at the single cell level. Beside the prevalence and amount of transcription, also the distributionacross the brain was considerably different. This is exemplified by looking at the distribution of the Htr1 and Htr2 families on the uniform manifold approximations and projection (UMAP, Figure 1b). The UMAP was color-coded according to neighborhood metadata. Neighborhoods were defined both by location and neurotransmitter (Supplementary Figure 1b, Table 1). When looking at the UMAP distributions for individual Htr, considerable differences were present also within each family of receptors (Supplementary Figure 2). We analyzed these differences grouping cells by neurotransmitter, neighborhoods or class. These categorizarions divided cells in a higlhy skewed manner (Supplementary Figure 1c), for example when looking at groups by neurotransmitter release, 3 groups (Glut, Gaba and unassigned) made up for almost the totality of cells (98.47%). Expectedly, the vast majority of cells was classified as excitatory (Glut, 50.79%) and around 1 every 5 cells was found to release GABA (20.62%). All the other neurotransmitter were found in less than 1% of the cells, in particular, 5-HT releasing neurons (Sero) were found in only 0.04% of the cells. Pattern of Htrs transcription across different neurotransmitter groups exhibited a relatevely high Pearson correlation coefficient (r=0.6±0.03). Sero and cholinergic neurons (Chol) showed the most distinct patterns of transcription with respectively r=0.32±0.04 and r=0.37±0.05 (Figure 1c). To better evaluate the uniqueness of Htrs RNA transcription per group we employed a Random Forest Classifier aiming at decoding the grouping variable from the Htrs transcription. Overall accuracy of the model was 37.13%. Reflecting the correlation analysis, the confusion matrix showed that Sero and Chol were among the groups with higher true positive (TP) rate (Sero=84.93%, Chol=42.55%). Cells not transcribing any neurotranmitter were also identified succesfully (81.24%). Moreover, Noradrenaline (Nora) and glycine (GABA-Glyc) releasing neurons were identified at considerable levels (Nora=45.16% and GABA-Glyc=50.64%). To understand the contribution of each Htr in each prediction we calculated the mean absolute SHAP (SHapley Additive exPlanations) values for each receptor and neurotransmitter {Lundberg, 2017 #2921; Lundberg, 2020 #2922}. The SHAP values in association with the mean prevalence enable us to understand the defining features of each group. Here we can see, for example, that the identification of Sero neurons is determined mainly by transcription of Htr1a and Chol neurons by Htr4 and Htr5b. Crucially, absence of transcription can also contribute to the classification, e.g., cells not transcribing any neurotransmitter are identified mainly by absence of any Htr. When looking at different neighborhoods the accuracy of the model was 51.75%. The model could differentiate best the NN-IMN-GC, TH-EPI-Glut and Pallium-Glut groups (NN-IMN-GC=73.78%, TH-EPI-Glut=65.57% and Pallium-Glut=57.15%, Supplementary Figure 3a). NN-IMN-GC includes all the cells not releasing any neurotransmitter, their classification is therefore predictably influenced by absence of any Htr. On the other hand, TH-EPI-Glut cells were characterized by the unique combination of high transcription of Htr7 and low transcription of Htr2a and Htr4, Pallium-Glut cells, instead, exhibited relatively low levels of Htr2c and Htr7. Notably, Htr7 and Htr1f seemed to follow opposite gradients across neighboroods.Across classes, differences in Htrs transcription were more striking (Figure 1d). 7 groups could be identified with a TP rate >40%: 04 DG-IMN Glut, 05 OB-IMN GABA, 09 CNU-LGE GABA, 18 TH Glut, 22 MB-HB Sero, 32 OEC, and 34 Immune (Supplementary Figure 3b). 04 DG-IMN Glut were charachterized by high transcription of Htr4 and absence of the usually prevalent Htr2c. Similarly, 05 OB-IMN GABA cells showed virtual absence of Htr2c as well as low Htr4 and high Htr1f transcription; 09 CNU-LGE GABA cells showed high Htr1b and low Htr7/Htr1a; 17 MH-LH Glut exhibited high levels of Htr5b and Htr4; 18 TH Glut showed high levels of Htr7 and virtual absence of Htr4; 22 MB-HB Sero, miroring the results showed by Sero neurons, were charachterized by high levels of Htr1a; at last, 34 Immune cells were identified by absence of any Htr transcription. The use of only Htrs transcription reached a unexpected 15.49% accuracy in decoding classes. Correlation between Htrs transcription across the totality of cells ranged from -0.03 (Htr1f-Htr3a) to 0.311 (Htr4-Htr2c). Considerable correlation was also found for the Htr7-Htr2c (r=0.264) and Htr1f-Htr2a (r=0.212) pairs (Figure 1e). Interestingly, correlation patterns were not stable across neighboroods (Supplementary Figure 4a). For example, Pallium-Glut exhibited a negative correlation between Htr4-Htr2a not evident from the entire dataset. Of note, TH-EPI-Glut showed the highest absolute correlation across all neighboroods with r=0.609 between Htr5b-Htr4 and a unique negative correlation between Htr4-Htr7. To explore the underlying causes of the correlations we analyzed colocalization between Htrs using the same stringent threshold used by the original authors to determine neurotransmitter transcription {Yao, 2023 #2828}. Across the entire dataset we observed that the most transcribed genes, Htr1f and Htr2c, were regurarly transcribed whenever the RNA of any other Htr was detected (Figure 1f). This was a driving factor for correlation. Looking more in detail across neighborhoods, also here we noticed important differences, mainly explainable by differential prevalence of Htrs in each neighborhoods. 86.41±1.69% of Htr-transcribing cells exhibits at least 2 Htrs (Figure 1g), therefore, only in a minority of cases a cell was found to transcribe uniquely one Htr (25.78% of the totality of cells). Surprisingly, 22.88±1.9% of Htr-transcribing cells transcribed at least 5 Htrs. The extensive transcription of different Htr families within the same cell point at the complexity of the 5-HT system even at the single cell dimension. To facilitate an understanding of the downstream cellular effects of 5-HT we aggregated receptors according to their main intracellular effector. We aggregated Htr1 and Htr5 due to their inhibitory effect (cAMP decrerase); Htr4, Htr6 and Htr7 because of the shared downstream effect of increasing cAMP; Htr2 is the only one that causes an Ca2+ increase while Htr3 is the only ionotropic receptor. For each cell we determined the principal pathway activated by 5-HT by analyzing the amount of RNA for each Htr and, afterwards, we grouped cells across different neighborhoods (Figure 1h). Ht3 were present only in a small minority of cortical inhibitory neurons. In the telencephalon, the absolute majority of both Pallium-Glut and Subpallium-Gaba cells were linked to Htr1/5, and around one quarter of cells instead featured Htr2 as primary effector. Subcortical cells exhibited a more balanced partition without any absolute majority and a considerable presence of Htr4/6/7. In the following sections we will take a deeper look at Htrs grouped by effector and we will take advantage of the spatial information provided by the MERFISH dataset of {Zhang, 2023 #2887} regarding 9 Htrs.

**Htr1 & Htr5**

Receptors belonging to these two families have an inhibitory effect on the host cell, they are coupled to Gᵢ and cause a downstream decrease of cAMP and activation of GIRK channels {Sharp, 2020 #2888; McCorvy, 2015 #2889}. Htr1a RNA have a stable prevalence of ≈10% across neighborhoods in the RNA-seq dataset (excluding non-neuronal cells and immature neurons), with virtual absence in the TH-EPI-Glut group (Figure 2a). Htr1a co-localized most frequently with Htr1f, Htr2c and Htr2a (Figure 2b) and only in a minority of cases was transcribed alone (<10%). transcription across classes was highly correlated between the RNA-seq and MERFISH datasets (Figure 2a) and show an almost perfect proportional relationship. Highest transcription was found in 5-HT neurons of the mid- and hindbrain (class 22 MB-HB Sero, Figure 2c), nonetheless, cortical excitatory neurons (01 IT-ET Glut), like in the majority of Htrs, contained the higher absolute number of cells transcribing Ht1a. Subclasses located in the hippocampus (HPF) contained most of the cells transcribing Htr1a (see online visualizer, 'Overview genes by class'). To pinpoint the spatial location we first identified the clusters highly enriched with Htr1a RNA with a threshold of 70%, i.e., to be classified as enriched at least 70% of cells in a cluster must express the receptor. Only 6.52% of Htr1a transcribing cells were contained in enriched clusters, pointing at a relatively low importance in the clustering algorithm. Looking at the spatial distribution across divisions, the highest prevalence was found in the pallidum (PAL) and HPF, two telencephalic regions (Figure 2d). At a more granular level, 5 of the top 10 structures by prevalence belonged to the raphe nuclei: dorsal nucleus raphe (DR), nucleus raphe obscurus (RO), nucleus raphe pallidus (RPA), nucleus raphe magnus (RM) and superior central nucleus raphe (CS). The high levels of Htr1a transcription in the raphe nuclei is reflection of the high prevalence in Sero neurons outlined above, the raphe nuclei contain the vast majority of Sero neurons of the brain. The hippocampal structure exhibiting the higher prevalence were the medial entorhinal cortex (ENTm) and the area prostata (APr) while The medial septum nucleus (MS) and the diagonal band nucleus (NDB), two structures linked to generation of theta waves {Winson, 1978 #2908} and contaiing Chol neurons, contributed substantially to the transcription in PAL. Notably, All these results confirms previous reports of Htr1a expression in the raphe {Haj-Dahmane, 1991 #2924;Sprouse, 1987 #2923}, ENTm {Schmitz, 1995 #2925; de Filippo, 2021 #1086} and MS {Kia, 1996 #2926}. Levels of of transcription were stable across the anterior-posterior axis like in most other Htrs (Figure 2e-f).   
Htr1b exhibited a more diverse pattern of transcription across neighboroods (Figure 3a) ranging from 10 to 30%. Highest prevalence was observed in the MB-HB-Glut-Sero-Dopa group, i.e., glutamatergic, serotonergic and dopaminergic neurons located in midbrain and hindbrain. Colocalization showed a similar pattern compared to Htr1a (Figure 3b) and also here only a minority of cells transcribed Htr1b alone (<10%). Looking at transcription across classes, the 09 CNU-LGE GABA class showed the highest prevalence (58.06%) closely followed by 22 MB-HB Sero (53.73%) (Figure 3c). High transcription in 09 CNU-LGE GABA was in sharp contrast with Htr1a that showed only minimal transcription in this class (1.61%). Also in this case, 01 IT-ET Glut exhibited the highest absolute number of Htr1b expessing cells, specifically cells in the nucleus of the lateral olfactory tract (NLOT, see online visualizer, 'Overview genes by class'). 17.48% of Htr1b transcribing cells belonged to highly enriched clusters and the striatum (STR) showed by far the highest prevalence with >30% (Figure 3d), in line with previous reports {Maroteaux, 1992 #2928;Pommer, 2021 #2927}. Caudoputamen (CP), Nucleus accumbens (ACB), olfactory tubercle (OT), lateral septal nucleus (LSc) and the parabigeminal nucleus (PBG) all exhibited a prevalence of >20%. DR and RO of the raphe nuclei also exhibited considerable prevalence. Similarly to Htr1a, Htr1b seems to be specifically linked to Sero neurons, in line with this, they have been reported to mediate self-inhibition in these neurons {Hjorth, 1991 #2932}. Distribution across the antero-posterior axes reflected the high prevalence in STR (Figure 3e-f).   
Htr1d was transcribed at a much lower level, never exceeding 7% prevalence in any neighborhood (Supplementary Figure 5a). It colocalized at highest levels with Htr2c and Htr1f (Supplementary Figure 5b) and only rarely was transcribed alone (<5%). Similarly to Htr1b, transcription was highest in 09 CNU-LGE GABA and 22 MB-HB Sero (Supplementary Figure 5c). Notably, 09 CNU-LGE GABA exhibited the highest absolute number of cells surpassing 01 IT-ET Glut. Only a small minority of Htr1d transcribing cells belonged to enriched clusters (2.08%). The paraventricular nucleus of the thalamus (PT and PVT) showed the highest prevalence at only >4% (Supplementary Figure 5d-e-f).   
Htr1f, surprisingly, showed the highest levels of transcription of all Htr in the RNA-seq dataset. Higher prevalence was found in the Pallium and Subpallium groups (Figure 4a), reaching ≈50%. Other groups showed a prevalence of 30-40% with TH-EPI-Glut at ≈20% (Figure 4a). Htr1f was found to colocalize the most with Htr2a and Htr2c (Figure 4b). In 30% of cases Htr1f was the only Htr transcribed in a cell and colocalization decreased linearly with the number of cotranscribed Htrs (Figure 4b). Notably, the slope of the linear regression between values provided by RNA-seq and MERFISH was significantly lower pointing at a difference absolute prevalence prer class (Figure 4c). The two datasets are, however, still highly correlated, with 66% of shared variability. This was the case also for other Htr2a, Htr2c and Htr4. Htr1f was broadly transcribed across almost all classes, including some non-neuronal cells, pineal gland cells were a notable exception. In absolute numbers, cortical glutamatergic cells showed the highest transcription. Various subclasses located in l5, claustrum (CLA) and HPF exhibited prevalence >50% (see online visualizer, 'Overview genes by class'). Spatial distribution showed a peculiarly asymettric pattern with transcription concentrated in the most anterior regions. Highest transcription was observed in STR, olfactory areas (OLF) and the cortical subplate (CTXsp) reaching >20% (Figure 4d). Specifically, the highest transcription was observed in nucleus accumbens (ACB) and olfactory tract (OT), similarly to Htr1b. The accessory olfactory bulb (AOB) was the OLF structure with the highest prevalence. CLA and the endopiriform nucleus (EPd), on the other hand, were the CTXsp structure exhibiting the highest prevalence. Interestingly, in the CTXsp, transcription in Glut and Gaba neurons was anticorrelated. High prevalence in GLut neurons corresponded to lower prevalence in Gaba and vice versa. In CLA and EPd HTR1f was transcribed mainly in Glut neurons, while in the amygdala (LA, BLA, BMA) in GAba neurons predominantly. Isocortex and HPF also exhibited considerable transcription both in excitatory and inhibitory neurons. The amount of RNA transcription per cell was not linear, with a clear peak in the frontal olfactory areas (Figure 4e-f). High transcription of Htr1f in this region was previously observed using immunohistochemistry {Bruinvels, 1994 #2929}. The broad transcription of Htr1f observed in the RNA-seq dataset across the entire telencephalon is in line with earlier reports {Vila-Pueyo, 2018 #2933}.   
Both Htr5a and Htr5b were not included in the MERFISH dataset, therefore we do not have any direct spatial visualization of their transcription. Htr5a was transcribed at 8-16% prevalence across all neighborhoods (Supplementary Figure 6a) and colocalized the most with Htr1f, Htr2c and Htr2a (Supplementary Figure 6b). transcription was broadly distributed across many classes, altough only at lower levels compared to other Htrs (Supplementary Figure 6c). Only one cluster was considered enriched with Htr5a in the entire RNA-seq dataset, 3453 PAG-PPN Pax5 Sox21 Gaba. This cluster was located mainly in the midbrain reticular nucleus (RR, Supplementary Figure 6d-e). Htr5b was transcribed at a much lower level (Supplementary Figure 7a), with a maximum of ≈%5 in TH-EPI-Glut. Surprisingly, even if its overall prevalence was much lower then Htr5a, 10 clusters were found to be enriched in Htr5b. This receptor was transcribed at considerable levels only in the 17 MH-LH Glut class (≈50% prevalence). This was caused by high levels of transcription in the medial habenula (MH, Supplementary Figure 7d-e), a structure involved in the response to stress and fear {Chou, 2016 #2913;Soria-Gomez, 2015 #2910;Winson, 1978 #2908;Yamaguchi, 2013 #2909}. Some transcription was also evident in the posterior part of the brain, specifically in the inferior olivary complex (IO), a structure strongly linked to cerbellar Purkinje cells {Loyola, 2023 #2914}. This transcription was driven by a single supertype, 253 IO Fgl2 Glut.

**Htr2**

The Htr2 family is mainly linked to Gq/11 and causes excitation by increasing intracellular Ca2+. Htr2a, famous for being instrumental in mediating the effects of psychedelics {Nichols, 2016 #854}, is found across the brain with highest prevalence in telencephalic neighborhoods, Pallium-Glut and Subpallium-GABA (Figure 5a). Colocalization was highest with Htr1f and Htr2c (Figure 5b). Considerable transcription (≈40%) was found in 01 IT-ET Glut, 07 CTX-MGE GABA and 16 HY-MM Glut classes (Figure 5c). Interestingly somatotatin (Sst) neuron belonging to 07 CTX-MGE GABA, while exhibiting a relatively low prevalence at the subclass level, contained various clusters with >70% prevalence {De Filippo, 2024 #2904}. Htr2a was also prevalent across many other classes across the whole brain. 01 IT-ET Glut exhibited by far the highest absolute number of neurons transcribing Htr2a, specifically in subclasses of l5 and CLA, resembling Htr1f (see online visualizer, 'Overview genes by class'). Interestingly, l5 extratelencephalic (ET) neurons exhibited considerable more Htr1f then Htr2a RNA. CTXsp showed the highest prevalence, reaching >12% (Figure 5d). Isocortex and STR exhibited both ≈5% prevalence. At a structure level, two structures belonging to the mammillary complex (dorsal premammillary nucleus, PMd and tuberomammillary nucleus,TMd) were in the top ten by prevalence. The mammillary complex has been linked to Alzheimer´s disease {Huang, 2023 #2915}, and memory {Roy, 2017 #2916}. CLA and the EPd showed the highest absolute prevalence. Interestingly, CLA has been proposed to play an important role in mediating the effects of psychedelic compounds {Doss, 2022 #2917}. Prevalence in the STR was driven by the small bed nucleus (BA), a structure important for the integration of limbic and enviromental informations {Lebow, 2016 #2931}. Htr2a transcription in CLA and mammillary complex is in line with a previous report in monkey{López-Giménez, 2001 #2930}. Transcription in BA was not previously reported. Prevalence of Htr2a was highest in frontal regions of the brain, decaying linearly to virtula absence in the cerebellum (Figure 5e-f).   
Htr2b was found only in a minority of neurons and was not included in the MERFISH dataset. No cluster was found to be enriched with Htr2b. Interestigly, neurons belonging to the Pineal Glut class showed the highest prevalence at 7.34%, an observation never reported previously.(Supplementary Figure 8c).   
Htr2c was found at highest prevalence in the MB-HB-Glut-Sero-Dopa and Hy-EA-Glut-Gaba groups (Figure 6a). With the exception of Pallium-Glut, its prevalence was always >40%. Colocalization was highest with Htr1f, Htr4 and Htr7 (Figure 6b). Transcription was broadly distributed across many different classes, also subcortically (Figure 6c). Many classes exhibited a prevalence >60%. As usual cortical excitatory neurons exhibited the highest number of cells expressing Htr2c. Some subclasses in OLF, amygdala and retrosplenial cortex (RSP) exhibited >80% prevalence (see online visualizer, 'Overview genes by class'). The majority of cells transcribing Htr2c RNA belonged to enriched clusters. Highest prevalence was found in STR. Similarly to Htr1b, ACB, CP and OT exhibited the highest prevalence (Figure 6d-e-f). Isocortex prevalence derived from the unique transcription in excitatory neurons of the ventral part of the RSP, curiosly the area with lowest transcription of the otherwise highly prevlent, Htr1f. High prevalence was observed also in excitatory neurons of the anterior olfactory nucleus (AON), and piriform area (PIR and PAA). Excitatory neurons of the amygdala (LA and BLA) also exhibited high transcription. Htr2c RNA was found across a variety of structures also in the MB (non in Sero neurons), pons (p), medulla (MY) and cerebellum (CB).

**Htr4, Htr6 and Htr7**

These receptor are all connected to Gs {McCorvy, 2015 #2889}, leading to increasing cellular levels of cAMP. Htr4, similarly to Htr2c, showed highest prevalence (>40%) in the MB-HB-Glut-Sero-Dopa and Hy-EA-Glut-Gaba groups (Figure 7a). It colocalized the most with Htr2c and Htr1f (Figure 8b). Transcription across classes was broadly distributed, with many classes showing a prevalence >40% (Figure 7c). Highest prevalence was found in the 17 MH-LH Glut class, specifically in the Chol releasing neurons of this class located in TH. In absolute numbers, transcription in excitatory cortical neurons was comparable to other classes but still the highest, driven specifically by subclasses of CA1, CA2, CA3 and subiculum (see online visualizer, 'Overview genes by class'). Spatial distribution exhibited a peculiar pattern with high prevalence in one specific structure of the STR: OT (Figure 7d-e-f). A subclass of interneurons present in OT (060 OT d3 Folh1 Gaba) showed a >98% prevalence. PAL and HPF also exhibited relatively high prevalence (≈10%). Dentate gyrus (DG) granule cells (037 DG Glut) were one of the reason of the high prevalence. Excitatory cells of CA2, CA3 and indusium griseum (IG) also transcribed often Htr4 RNA.   
We do not have MERFISH information about the rarely transcribed Htr6 and no enriched cluster was present in the RNA-seq dataset. The 09 NU-LGE GABA class exhibited the highest prevalence with 7.73, still, the absolute majority of neurons transcribing the RNA of this gene were excitatory cortical neurons (Supplementry Figure 11c).   
Conversely, Htr7 was transcibed in >10% of the totality of cells. It reached ≈60% in the TH-EPI Glut group, and considerable amounts (≈40%) in MB, HB and HY groups (Figure 8a). Colocalization was the highest with Htr2c and Htr1f (Figure 8b). transcription was broadly distributed across classes present in HY, MB and TH (Figure 8c). It colocalized the most with Htr2c, tr1f and Htr4. Htr7 was broadly transcribed across classes, especially in subcortical structures. Peak prevalence was found in 10 LSX GABA, 16 MY MM Glut and 18 TH Glut with >60% (Figure 8c). Cortical expression in excitatory neurons is driven primarily by subclasses in CA2 and l2 ENT (see online visualizer, 'Overview genes by class'). Htr7 enriched clusters were located mainly in HY and TH (Figure 8d). At a structure level, the parafascicular (PF) and paraventricular nucleus (PVT) of TH showed the highest prevalence (>30%).

**Htr3**

The Htr3 family is the only ionotropic Htr and it causes direct excitation by allowing the influx of cations. The Htr3a subunit is required for the formation of a functional channel {Maricq, 1991 #2918} and can form functional homopentameric receptors {Walstab, 2010 #2919}. Heteromeric receptors containing Htr3b have an increased channel conductance and different selectivity {Davies, 1999 #2920}. Htr3a istranscribed almost uniquely in the Subpallium-Gaba neighborood, with a prevalence of ≈8% (Supplementary Figure 10a), specifically in the 06 CTX-CGE GABA class (Supplementary Figure 10c). It is one of the few Htr, together with Htr3b and Htr1d, that is not transcribed the most in absolute numbers in 01 IT-ET glut. It colocalizes mainly with Htr2c and Htr7 (Supplementary Figure 10b). This Htr was mainlytrancribed in OLF, CTXsp, HPF and Isocortex (Supplementary Figure 10d) and is most prevalent in the anterior part of the brain, altough, puzzingly, with slightly lower level of transcription (Supplementary Figure 10e-f). Htr3b was not included in the MERFISH dataset and no cluster was found to be enriched with this receptor. Htr3b was the leasttranscribed Htr gene in the entire dataset. Similarly to Htr3a, its transcription was delimited to the 06 CTX-CGE GABA class (Supplementary Figure 11c).

# Discussion

In this work we described the main transcriptional features of each Htrs across the entire brain leveraging two datasets provided by {Yao, 2023 #2828} and {Zhang, 2023 #2887}. We found that Htrs RNA is transcribed in around 2 every 3 cells and 6 Htrs were transcribed in >10% of cells, with Htr1f reaching a peak of 34.26%. Htrs patterns of transcription can be used to decode the identity of cells grouped by neurotransmitter, neighborhoods and classes at an above chanche level. Surprisingly, it was more common to detect multiple Htrs within a cells in comparison to single Htrs. This points at the great complexity of the 5-HT system even at the a unicellular level.We can recapitulate our results regarding each Htr by summarizing the defining feature of each receptor. Htr1a is expressed in an important fraction of Sero neurons of the raphe and some HPF excitatory neurons. Htr1b is expressed in a many inhibitory striatal neurons and raphe Sero neurons. Htr1d, similarly to Htr1b, is expressed in the striatum, although at much lower levels. Htr1f is widely expressed in telencephalic structures,especially the Isocortex, with a peak in frontal olfactory structures. Htr2a is prevalent in glutamatergic cells of the cortical subplate (CLA and EPd) and the mammillary bodies (TMd, PMd), and hippocampal interneurons. Htr2b is rarely transcribed and is present in some neurons of the pineal gland. Htr2c is broadly transcribed, especially in the STR, excitatory neurons of the amygdala (LA, BLA and BMA) and RSPv, OLF neurons and structures in MB, P, MY and CB. Htr3a and Htr3b are uniquely observed in cortical gabaergic neurons of the 06 CTX-CGE GABA class. Htr4 is transcribed at high levels in the OT, excitatory cells of the hippocampus proper and DG, and Chol neurons of the TH (17 MH-LH Glut). Htr5a is transcribed at low levels with only one enriched cluster in the MB. Htr5b is also transcribed only in few cells, specifically in Chol neurons of the TH. Htr6 does not feature any enriched cluster, some cells in CA3 transcribed this Htr. Htr7 is widely transcribed in subcortical structures, especially in some TH nuclei (PF, PVT, IAD and PT), the mammillary complex (MM and PMd), the lateral septal nucleus (LSv) and the fasciola cinerea of the HPF. Our analysis is in no way exhaustive and it is limited in scope by the costraints of a traditional scientific articles. To bypass this limit and, at the same time, provide the ability to explore the 5-HT transcription landscape at different depth of the atlas classification we provide a custom online visualizer. The visualizer enbles the exploration of: Htrs transcription in the MERFISh dataset; the prevalence of each Htr across neighborhoods,class, subclass, supertype and clusters; an overview of Htrs prevalence across classes and subclasses; and an overview of Htrs prevalence across all brain divisions and structures optionally grouped by neurotransmitter release.

# Materials and Methods

**Online visualizer**

The online visualizer was built in python using Matplotlib, Holoviews and Panel. It is deployed and accesible online on the Hugging Face portal https://huggingface.co/organizations/RDFm982367h7ndq/share/sJrEwlxfHoaNuXuQcqXjuZhbqkGbuyqxxa

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# Data and materials availability

All the code used to process the dataset is available at https://github.com/RobertoDF/Transcriptomics-5-HT, pre-computed data structures can be downloaded at 10.6084/m9.figshare.20209913. All figures and text can be reproduced using code present in this repository. Access to the original datasets is provided by the Allen Institute at https://portal.brain-map.org/atlases-and-data/bkp/abc-atlas.

# Contributions

Conceptualization, data curation, formal analysis, investigation, visualization: RDF. Writing - original draft: RDF. Writing - review & editing: RDF, DS. Funding acquisition: DS.

# Figures

**Figure 1. Overview of Htrs translation in the RNA-seq dataset.**

(A) Heatmap showing absolutwe number of cells expressing each Htrs. Inset shows the same information in percentage of the total. (B) UMAP representation color-coded by neighborhood metadata (left), Htr1 (middle) and Htr2 (right) expression. (C) Htr expression prevalence in cells grouped by neurotransmitter release (top). Confusion matrix of the multi-label random forest classifier showing true label on y axis and predicted label on x axis (middle). Matrix of absolute SHAP values for each group and receptor (bottom). (D) Htr expression prevalence in cells grouped byclass. (E) Htrs expression correlation matrix. (F) Htrs colocalization matrix. Each dot represents the percentage of colocalization of gene on x axis in cells expressing gene on y axis. (G) Top: Percentage of cells expressing the number of Htrs on the x axis. Percentage of cells expressing the gene on x axis expressing at least another Htrs gene (middle) or at least other 4 Htrs (bottom). (H) Pie charts representing the proportion of principal Htrs grouped by intracellular effector for each neighborhood. Principal effector was identified by summing the expression of Htrs. Each number represents the number of cells in thousands.

**Htr1a transcription**

(A) On the left, Htr1a prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr1a RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr1a RNA (left). Number of Htrs RNA detected in cells expressing Htr1a RNA (right). (C) Prevalence of Htr1a RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr1a RNA by class ranked in descending order (top ten). (D) Prevalence of Htr1a RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr1a RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr1a RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

**Htr1b transcription**

(A) On the left, Htr1b prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr1b RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr1b RNA (left). Number of Htrs RNA detected in cells expressing Htr1b RNA (right). (C) Prevalence of Htr1b RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr1b RNA by class ranked in descending order (top ten). (D) Prevalence of Htr1b RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr1b RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr1b RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

**Htr1d transcription**

(A) On the left, Htr1d prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr1d RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr1d RNA (left). Number of Htrs RNA detected in cells expressing Htr1d RNA (right). (C) Prevalence of Htr1d RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr1d RNA by class ranked in descending order (top ten). (D) Prevalence of Htr1d RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr1d RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr1d RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

**Htr1f transcription**

(A) On the left, Htr1f prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr1f RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr1f RNA (left). Number of Htrs RNA detected in cells expressing Htr1f RNA (right). (C) Prevalence of Htr1f RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr1f RNA by class ranked in descending order (top ten). (D) Prevalence of Htr1f RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr1f RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr1f RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

**Htr2a transcription**

(A) On the left, Htr2a prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr2a RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr2a RNA (left). Number of Htrs RNA detected in cells expressing Htr2a RNA (right). (C) Prevalence of Htr2a RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr2a RNA by class ranked in descending order (top ten). (D) Prevalence of Htr2a RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr2a RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr2a RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

**Htr2b transcription**

(A) On the left, Htr2b prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr2b RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr2b RNA (left). Number of Htrs RNA detected in cells expressing Htr2b RNA (right). (C) Prevalence of Htr2b RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr2b RNA by class ranked in descending order (top ten). (D) Prevalence of Htr2b RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr2b RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr2b RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

**Htr2c transcription**

(A) On the left, Htr2c prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr2c RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr2c RNA (left). Number of Htrs RNA detected in cells expressing Htr2c RNA (right). (C) Prevalence of Htr2c RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr2c RNA by class ranked in descending order (top ten). (D) Prevalence of Htr2c RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr2c RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr2c RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

**Htr3a transcription**

(A) On the left, Htr3a prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr3a RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr3a RNA (left). Number of Htrs RNA detected in cells expressing Htr3a RNA (right). (C) Prevalence of Htr3a RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr3a RNA by class ranked in descending order (top ten). (D) Prevalence of Htr3a RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr3a RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr3a RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

**Htr3b transcription**

(A) On the left, Htr3b prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr3b RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr3b RNA (left). Number of Htrs RNA detected in cells expressing Htr3b RNA (right). (C) Prevalence of Htr3b RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr3b RNA by class ranked in descending order (top ten). (D) Prevalence of Htr3b RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr3b RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr3b RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

**Htr4 transcription**

(A) On the left, Htr4 prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr4 RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr4 RNA (left). Number of Htrs RNA detected in cells expressing Htr4 RNA (right). (C) Prevalence of Htr4 RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr4 RNA by class ranked in descending order (top ten). (D) Prevalence of Htr4 RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr4 RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr4 RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

**Htr5a transcription**

(A) On the left, Htr5a prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr5a RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr5a RNA (left). Number of Htrs RNA detected in cells expressing Htr5a RNA (right). (C) Prevalence of Htr5a RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr5a RNA by class ranked in descending order (top ten). (D) Prevalence of Htr5a RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr5a RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr5a RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

**Htr5b transcription**

(A) On the left, Htr5b prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr5b RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr5b RNA (left). Number of Htrs RNA detected in cells expressing Htr5b RNA (right). (C) Prevalence of Htr5b RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr5b RNA by class ranked in descending order (top ten). (D) Prevalence of Htr5b RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr5b RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr5b RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

**Htr6 transcription**

(A) On the left, Htr6 prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr6 RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr6 RNA (left). Number of Htrs RNA detected in cells expressing Htr6 RNA (right). (C) Prevalence of Htr6 RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr6 RNA by class ranked in descending order (top ten). (D) Prevalence of Htr6 RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr6 RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr6 RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

**Htr7 transcription**

(A) On the left, Htr7 prevalence across neighborhoods with squared Pearson correlation coefficient (R²) between RNA-seq and MERFISH dataset.. On the right, amount of Htr7 RNA detected using RNA-seq (top) and MERFISH (bottom). (B) Amount of colocalization with each Htrs by cells expressing Htr7 RNA (left). Number of Htrs RNA detected in cells expressing Htr7 RNA (right). (C) Prevalence of Htr7 RNA across all classes of cells in RNA-seq and MERFISH dataset. Inset represents the linear regression between the two datasets. On te right, absolute number of cells expressing Htr7 RNA by class ranked in descending order (top ten). (D) Prevalence of Htr7 RNA across divisions (left) and structures (right). Inset represents the proportion of cells expressing Htr7 RNA that belongs to enriched clusters. (E) Prevalence (top) and average amount of RNA expression across the antero-posteroir axis. (F) Expression of Htr7 RNA detected by MERFISH in 4 representative slices. Border color represents the position on the antero-posterior axis.

# Supplementary Figures

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**Htr1a**